BACTERIAL LEAF SPOT OF SQUASH

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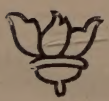

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BACTERIAL LEAF SPOT OF SQUASH[1]

By MARY K. BRYAN

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INTRODUCTION

Bacterial leaf spot of squash first came to the attention of the writer in 1925 when specimens were sent to E. F. Smith from Ithaca, N. Y., by C. D. Chupp, who wrote concerning the infected plants:

> The disease started in the center of a small planting of Hubbard squash and is spreading rather rapidly in all directions. It kills the foliage and seems to be very virulent.

In reply to a request for further information he wrote:

> The plants were not killed entirely, but many of the leaves were so badly blighted that the plants looked almost black. Apparently the bacterium must have been carried in the seed, since none of the Hubbard squash were grown in that vicinity, and so far as I know Hubbard squash had never been planted in that garden before.

It was at first suspected that this was a new host for *Bacterium lachrymans*, but the isolation of a yellow bacterium that was proved pathogenic to squash by inoculation disproved this theory. In a brief description of the organism [2] the name *Bact. cucurbitae* was proposed. A short account also appeared in the Yearbook of the United States Department of Agriculture.[3] The present paper is intended to give a more complete description of the pathogene and of its effect on its hosts.

GEOGRAPHICAL DISTRIBUTION

Since its discovery in New York, bacterial leaf spot of squash has been found in Maryland, the District of Columbia, Georgia, and South Carolina. In New York it has been reported on Hubbard squash only, but in Maryland it was found on Hubbard squash and Boston Marrow (*Cucurbita maxima*), pumpkin, and Golden Summer Crookneck squash (*C. pepo condensa*). In the District of Columbia, Georgia, and South Carolina it has been found only on Golden Summer Crookneck squash.

SYMPTOMATOLOGY

Lesions are confined chiefly to the leaves, but occasionally young stems and petioles are attacked. No infections have been found or produced by inoculation on fruits. Leaf spots are conspicuous, especially in the early stages, for the wide, bright-yellow halo that surrounds them.

Infections are first apparent two or three days after spray inoculation. At that time they show on the lower surface as minute water-soaked areas penetrating to the upper surface as indefinite yellow

[1] Received for publication Sept. 10, 1929; issued February, 1930.

[2] BRYAN, M. K. BACTERIAL LEAF SPOT ON HUBBARD SQUASH. Science (n. s.) 63: 165. 1926.

[3] BRYAN, M. K. SQUASH OF HUBBARD VARIETY ATTACKED BY NEW LEAF SPOT. U. S. Dept. Agr. Yearbook 1927: 599–600., illus. 1928.

areas. They enlarge rapidly, so that by the fourth or fifth day there is a definite round or angular, thin, brown, translucent center having on the upper surface a wide yellow halo (fig. 1) and on the lower surface either a water-soaked margin or no differentiation beyond the sunken spot. With further enlargement the spots are angular and restricted by the veins. Single isolated spots may reach a diameter of 6 or 7 mm.

Large dead areas are produced by the coalescing of crowded spots, as shown on the lower part of the inoculated leaf. (Pl. 1.) Here each spot is delimited by the veins which separate it from adjoining spots. The whole area is thin, dry, translucent, and mottled light and dark brown. The same patchwork effect is seen in the original material from Ithaca, N. Y. (Pl. 2.) Although papery, thin, and dry, the spots do not tear and drop out as in angular leaf spot of cucumber.

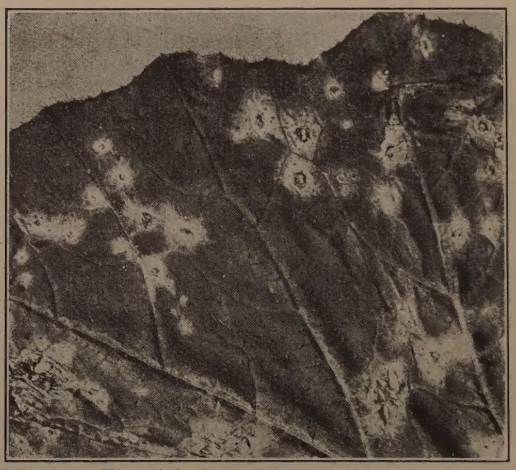


FIGURE 1.—Young infections on a Hubbard squash leaf from a hothouse plant. Collected seven days after spraying with *Bacterium cucurbitae*. × 1

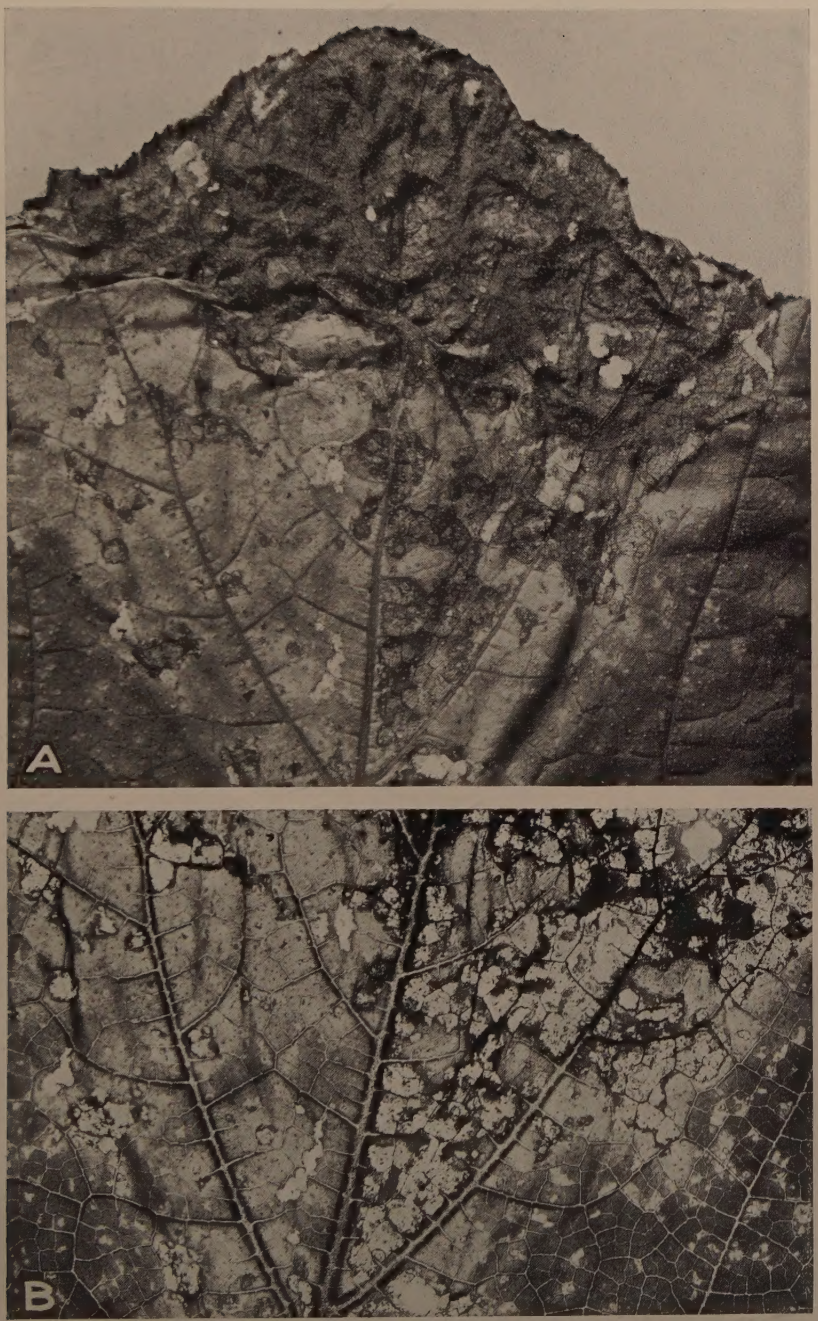
Large dead areas also form where injury to veins causes the shriveling of the tissues beyond. This is seen in the tip of the leaf shown in Plate 1, A. Spots and aggregates of spots may be distinguished within this dead, opaque area.

On shaded portions of leaves the spots may be dark gray instead of brown; the yellow halo is as conspicuous on such spots as on those exposed to direct sunlight.

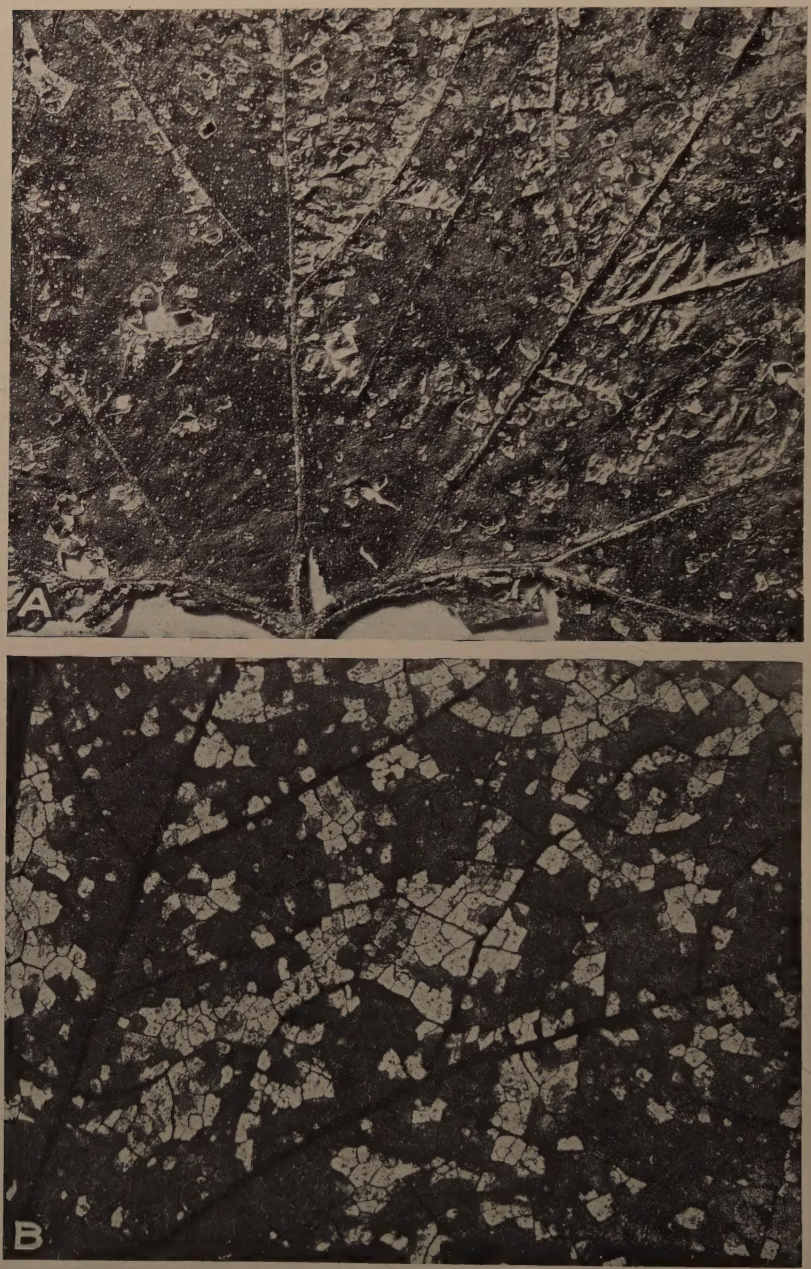
Occasionally young stems of summer squash are attacked (fig. 2) and cracking results. Very young plants may be killed by injury to the growing point when thus infected.

PATHOLOGICAL HISTOLOGY

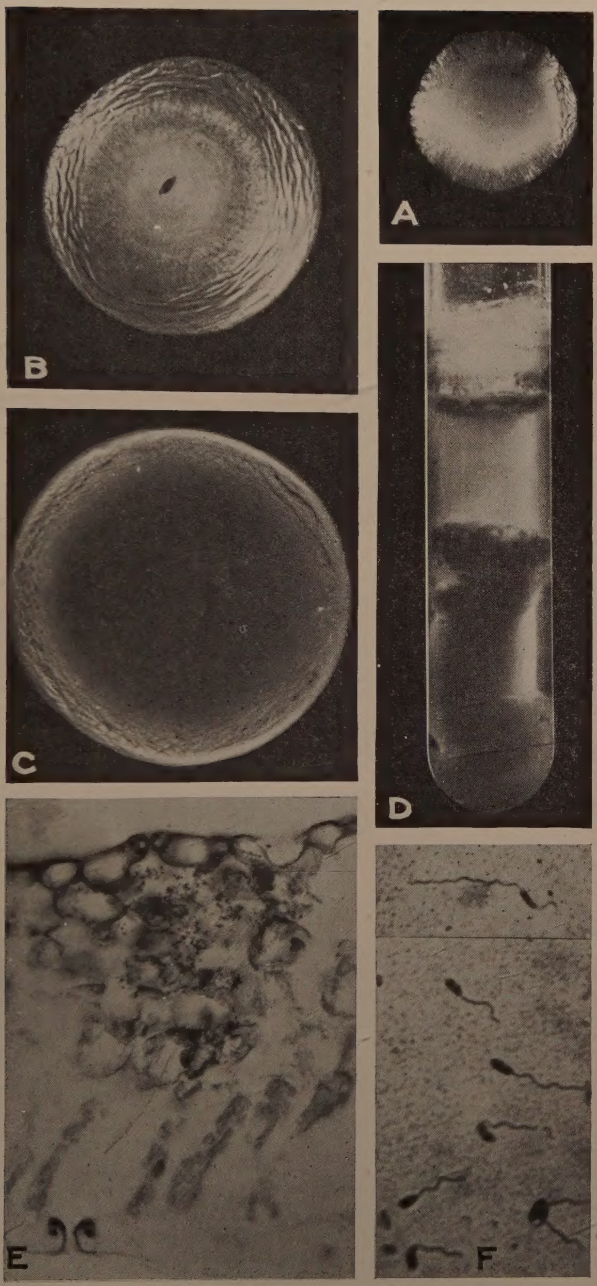
The bacteria enter the leaf by way of the stomata, which are very numerous on both leaf surfaces, especially on the lower one. Bacteria may be demonstrated in the substomatal chamber and penetrating half the thickness of the leaf on the second day (pl. 3, E), after inoculation by spraying. At that time the only outward indication of infection is a slightly water-soaked spot on the lower surface. By the fourth day bacteria have spread in all directions, filling the intercellular spaces of the loose parenchyma in the lower part of the leaf and wedging between the palisade cells to the upper epidermis. In stained sections cut at this stage no collapse has taken place. The cells of the infected area are turgid, but the cell walls in the invaded area take a deeper stain than those in the adjoining tissues. Very shortly after this stage the invaded tissues collapse. This collapse is much more evident on the lower surface than on the upper.



Secondary infections on a Hubbard squash leaf from a plant inoculated with *Bacterium cucurbitae*: A, Photographed by reflected light; B, by transmitted light. × ⅔



Dry leaf, showing natural infection by *Bacterium cucurbitae* on original Hubbard squash leaf from Ithaca, N. Y.: A, Photographed by reflected light; B, by transmitted light. × ⅔



A–C.—Agar-plate colonies of *Bacterium cucurbitae* taken by oblique transmitted light: A, 5 days old, × 5; B, 7 days old, × 5; C, 9 days old, × 7

D.—Milk culture, 4 weeks old

E.—Section of Hubbard squash leaf showing stomatal infection two days after inoculation by spraying with *Bacterium cucurbitae*. × 400

F.—*Bacterium cucurbitae*, showing flagella stained by Casares-Gil's stain. Note the extremely long flagellum on the uppermost rod. These occur frequently on this organism. × 940

Bacterial leaf spot should not be confused with the virus disease described as ring spot by Wingard.[4] In early stages of systemic ring spot on squash where the water-soaked or necrotic center is of pin-point size with a yellow halo, it might be difficult to distinguish the lesions from those of bacterial spot. Later stages could not be confused. On pumpkin, however, the spotting as shown in Wingard's[5] Figure 20 is very suggestive of bacterial spot, except that in the latter disease the spots do not follow the veins.

FIGURE 2.—Stem of young Golden Crookneck summer squash infected with *Bacterium cucurbitae*. ×1

INOCULATIONS

Stomatal infections are readily produced on Hubbard squash by spraying the plants in inoculation cages with a suspension of *Bacterium cucurbitae*. From two to three days elapse between the time of inoculation and the first appearance of lesions. The youngest completely or partially expanded leaves are most susceptible.

Good infections have been obtained in the manner just described on all the varieties of the genus Cucurbita on which the disease occurred in the field and also on Italian summer squash, Early White Bush Scalloped squash, Large Field pumpkin, and Sugar Pie pumpkin. Several varieties of *Cucurbita moschata* (Striped Cutshaw, Winter Crookneck, and Large Cheese pumpkin) have been successfully inoculated. Cucumis species, such as cucumber (*C. sativus*) and muskmelon (*C. melo*), appear immune. Very minute infections were once produced on cucumber, but they did not develop beyond pin-point size. Good infections have been obtained on watermelon (*Citrullus vulgaris*). (Fig. 3.)

Reisolations were made from spots on each of the successfully inoculated varieties, and the characteristic colonies thus obtained proved infectious on Hubbard squash.

[4] WINGARD, S. A. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. Jour. Agr. Research 37: 127–153, illus. 1928.
[5] WINGARD, S. A. Op. cit.

Seed inoculated with *Bacterium cucurbitae*, dried and planted, have yielded as high as 15 per cent infection, the disease sometimes showing first on the cotyledons and at other times on the first leaves.

Inoculations with *Bacterium cucurbitae* both by spraying on and pricking in the inoculum, on cotton, cabbage, string bean, Lima bean, and poppy, all of which are hosts of other yellow plant patho-

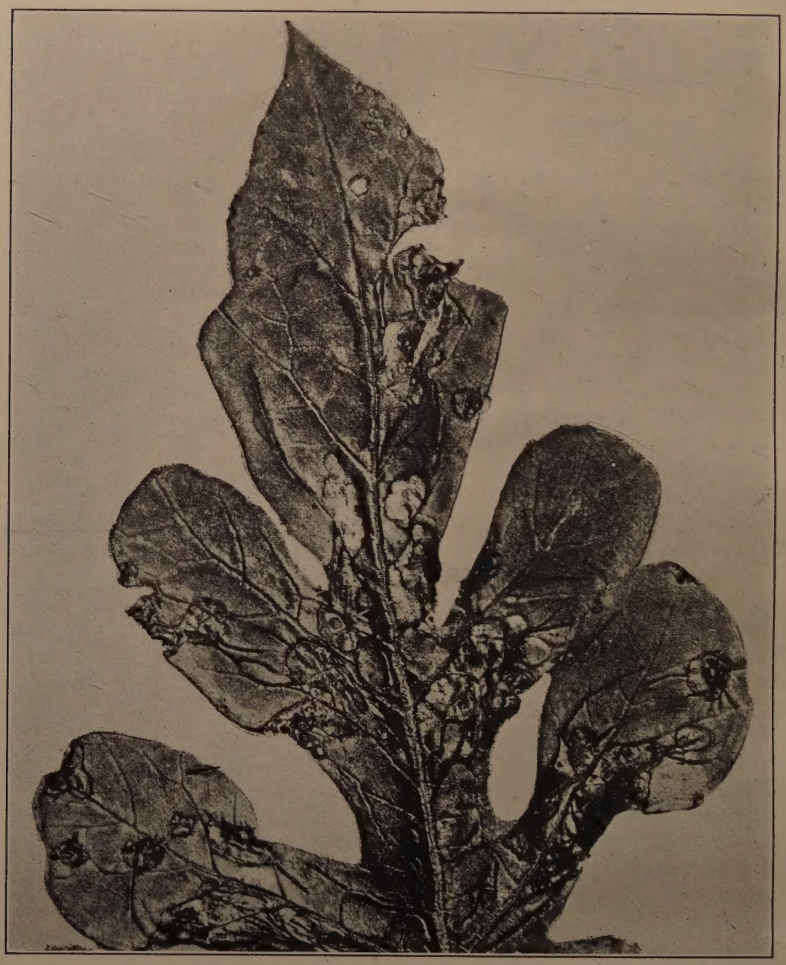


FIGURE 3.—Watermelon leaf five days after spraying with *Bacterium cucurbitae*. × 1¼

genes, have been entirely negative. Good infections resulted on Hubbard squash used as checks.

METHOD OF DISSEMINATION

The organism is apparently carried on the seed, but it does not appear to infect the soil. Although weather conditions favored infection, perfectly healthy Hubbard squash vines were grown on a plot in the District of Columbia where the disease had been very

severe the previous year. Further observations are needed to complete the evidence on these points. Spread in the field is facilitated by weather conditions, chiefly by rain. Potted plants of Hubbard squash, with several infected leaves each, set out in the ground early in July, developed vigorous healthy vines with no new infections during a long period of dry weather, but during a 10-day rain in August following this drought heavy infection spread rapidly through the patch and continued to spread with succeeding rains. Newly infected leaves appeared adjacent to old infections, and although squash bugs, cucumber beetles, and aphids were abundant on the vines, no infections were found where they fed on the leaves, at a distance from infected leaves. It would seem, therefore, that these insects do not spread the disease.

THE PATHOGENE

MORPHOLOGY

Bacterium cucurbitae is a short, polar-flagellate rod, 0.5μ to 1.3μ by 0.45μ to 0.6μ, usually with only one flagellum, which is frequently of unusual length. (Pl. 3, F.) It occurs singly, in pairs, or in short chains. Capsules are formed, but no spores.

REACTION TO STAINS

This organism stains readily with the usual bacterial stains, is definitely Gram negative, and is not acid fast.

CULTURAL CHARACTERS[6]

BEEF-AGAR PLATES.—Colonies on thinly sown plates are visible on the third day. They are less than 1 mm. in diameter, round, pale yellow, opalescent, and finely crosshatched or without markings. By the fifth day they are 3 or 4 mm. in diameter, showing by transmitted light an opaque center and a thinner margin, which may be either crosshatched or show concentric lines. (Pl. 3, A.) Some colonies are slightly umbonate. Later they may reach a diameter of 6 or 7 mm. and become decidedly umbonate. The concentric lines or crosshatching persists, and the opalescence remains very striking. (Pl. 3, C.) Sometimes fine radiating lines appear in the margin (pl. 3, A) or as a ring between the dense center and the thin margin. (Pl. 3, B.) The color, at first wax yellow, becomes mustard yellow.[7] The consistency is at first viscid and later butyrous. On plates 2 weeks old secondary growths of deeper color have formed in the colonies. Buried colonies are lenticular; bottom colonies thin, white, and granular with one or two concentric rings.

BEEF-AGAR SLANTS.—Growth is moderate, 2 to 6 mm. wide, mustard yellow, opalescent, and smooth with undulating margins. The consistency is viscid at first, but it becomes butyrous. Usually no crystals are formed, but occasionally a few small crystals appear just below the surface of the slant.

BEEF-EXTRACT AGAR.—Growth on beef-extract agar is deeper yellow, more restricted with less noticeable markings and less conspicuous opalescence than on beef-infusion agar.

BEEF BROTH.—Clouding is prompt but moderate. A rim of coarse pseudozoogloeae is formed, and in undisturbed cultures also a partial or complete pellicle of the same composition. Cultures 3 weeks old or more are clear or almost so and have a heavy yellow precipitate, which rises in a viscid swirl on shaking. Numerous small crystals lie in the precipitate.

POTATO-DEXTROSE AGAR.—On this medium growth is abundant, soon covering the entire surface with a heavy, butryous, pale-yellow layer of growth.

[6] Unless otherwise stated, all beef media were made with beef infusion and had a pH of 6.8 to 7.2.

[7] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912

POTATO CYLINDERS.—On steamed-potato cylinders growth is very heavy, butyrous, and bright yellow, covering the potato and filling the water with a dense yellow slime, eventually almost or completely submerging the potato, which shrinks with the destruction of the starch. Old cultures take on a more or less deep-brown tinge.

SYNTHETIC MEDIA.—In Cohn's solution growth is very feeble or absent. Very moderate growth occurs in Uschinsky's solution. In Fermi's solution cultures cloud moderately. In none of these media is there any rim, pellicle, precipitate, or fluorescence.

PHYSIOLOGY

LIQUEFACTION OF GELATIN.—In gelatin stabs at 20° C. liquefaction begins on the second day, is saucer shaped, becoming stratiform, and is only half complete in one month. At this stage there are an abundant yellow precipitate and a yellow rim, but the liquefied gelatin is practically clear. Liquefaction is complete in six to eight weeks.

LIQUEFACTION OF BLOOD SERUM.—Clearing of the serum begins on the fourth day, followed on the fifth by liquefaction, which extends throughout the slanted portion and slightly below; but neither process reaches the bottom of the tube.

FERMENTATION OF SUGARS.—The ability of the organism to ferment various carbohydrates was tested on peptone-free synthetic agar [8] with brom-cresol purple as an indicator. One per cent of each of the following was used: Sucrose, dextrose, lactose, maltose, galactose, levulose, glycerin, and mannit. Acid was formed from all except mannit, beginning on the seventh day in sucrose, dextrose, galactose, and maltose, and in nine days in lactose, glycerin, and levulose. The cultures never became acid throughout, the lower portions remaining pale purple.

When the same carbohydrates were tested in beef-extract agar with brom-cresol purple as indicator no acid was evident at any time; in the same beef-extract agar with the same carbohydrates and with phenol red as indicator, an alkaline reaction began within 24 hours and within 10 days had spread throughout the agar in all cultures.

No gas was formed in fermentation tubes from any of these carboyhdrates, nor was there any growth in the closed end.

HYDROLYSIS OF STARCH.—The starch of potato cylinders is almost completely destroyed and the shrunken cylinder submerged in the abundant yellow growth. On starch-agar plates a clear area 2 to 2.3 cm. wide around the streak of bacterial growth is produced within seven days.

RELATION TO FREE OXYGEN.—The organism is aerobic; it does not grow in the closed end of fermentation tubes and makes no growth in the lower half of agar stabs or in the lower part of shake-agar cultures.

NITRATE REDUCTION.—Nitrates are not reduced.

REACTIONS IN MILK.—No acid is formed in milk; peptonization sets in on the fifth or sixth day and is complete in eight days. Separation begins at about this time. At the end of three weeks the cultures present the appearance shown in Plate 3, D, with a heavy yellow rim above a layer of clear whey; below this is a heavy yellow precipitate on top of the soft curd, which has drawn away from the sides of the tube. Occasionally a few tyrosine crystals are formed. Reduction of methylene blue begins in three days and is complete in seven days. Reduction of litmus begins by the tenth day and is complete by the fourteenth day.

PRODUCTION OF AMMONIA.—A moderate amount of ammonia is produced in beef media.

PRODUCTION OF HYDROGEN SULPHIDE.—Hydrogen sulphide is produced in beef broth. Tests with strips of lead-acetate paper inserted in beef-broth cultures became a deep brown by the sixth day.

PRODUCTION OF INDOL.—No indol is produced.

TOLERATION OF SODIUM CHLORIDE.—Clouding is prompt and heavy in beef broth containing 1 and 2 per cent NaCl. It is retarded slightly in the presence of 3, 4, and 5 per cent. Growth in 5 per cent remains very moderate and contains many chains.

OPTIMUM pH FOR GROWTH.—The organism makes its best growth in pH 6.5 to 7.0. The limits for growth are pH 5.8 and pH 9.0.

TEMPERATURE RELATIONS.—The best growth takes place at 25° to 30° C., no growth occurs above 35°, and the thermal death point is 49°.

LONGEVITY ON CULTURE MEDIA.—Cultures of this organism are shorterlived on culture media than most pathogenes; even beef-agar stabs, when kept in the

[8] SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIC. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA . . . 48 p., illus. Geneva, N. Y. [1923.]

ice box, must be transferred every three or four months, as after six months without transfer most strains are dead.

TECHNICAL DESCRIPTION

Bacterium cucurbitae is a short rod 0.5 μ to 1.3 μ by 0.45 μ to 0.6 μ, with one polar flagellum, occurring singly, in pairs, or in short chains. It forms no spores, is not acid fast, and is Gram negative. On beef agar it forms round, yellow, opalescent colonies with internal striae. Its diastasic action is strong. It liquefies gelatin and blood serum slowly. Acid without gas is formed from sucrose, dextrose, lactose, maltose, galactose, levulose, and glycerin, but not from mannit. It does not form acid in milk, but reduces litmus and methylene blue in this medium. It does not reduce nitrates or form indol, but it does produce ammonia and hydrogen sulphide. Its optimum pH or growth is 6.5 to 7.0. Its optimum temperature is 25° to 30° C., its thermal death point being 49°.

SUMMARY

This paper describes a bacterial leaf spot which attacks summer and winter squashes and pumpkins. The disease can be transmitted to watermelon by inoculation, but it has not been found occurring on this plant in the field. It has not been found on cucumbers or muskmelons, and inoculations have failed to produce it on them.

The leaf spots are brown surrounded by a yellow halo, and they do not tear or drop out as do those of angular leaf spot of cucumbers. They often coalesce to form large dead areas.

The disease appears to be seed borne. The evidence at hand indicates that it does not live over winter in the field and is not transmitted by insects. It spreads rapidly in rainy weather.

A description is given of *Bacterium cucurbitae*, the causal organism.